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Full Length Research Paper

Assessment of somaclonal variation for salinity tolerance in sweet potato regenerated plants

Nadia Anwar*, Akira Kikuchi and Kazuo N. Watanabe

Gene Research Center, Graduate School of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Ten-noudai, Tsukuba, Ibaraki 305-8572, Japan.

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Genetic variation is the source for plant breeding. Somaclonal variation is genetic variation induced during tissue culture and also during ordinary growth *in vivo*, and occurs rather, often in sweet potato. The aim of the present study was to evaluate the degree of somaclonal variation in regeneration via somatic embryogenesis by phenotypic analysis under salinity stress condition and to assess the potential of somaclonal variation for development of salinity tolerant cultivar in sweet potato. The regenerated and control plants were evaluated under an established *in vitro* salinity screen system where media were supplemented with 0, 75, 150 and 200 mM of NaCl. The data for parameters (number of roots, length of roots, leaf and root condition) was recorded in three repeat tests. Data analysis suggested a significant variation in salinity tolerance among regenerated and control plants that proved the occurrence of somaclonal variation in regenerated plants. Despite none of the regenerated line was selected as a salt tolerant line, present study shows that regenerated plants exhibited somaclonal variation that can be utilized for selection of desired traits in sweet potato.

Key words: Sweet potato, regeneration, somaclonal variation, salinity tolerance.

INTRODUCTION

Sweet potato (*Ipomoea batatas* L. Lam.) is an asexually propagated root crop of family *Convolvulaceae* and is cultivated worldwide as a valuable source of food, animal feed and industrial raw material (Woolfe, 1992). Sweet potato is the fifth most important food crop in developing countries where over 95% of global sweet potato crop is produced. Nearly half of the sweet potatoes produced in Asia are used for animal feed whereas most of the crop cultivated in Africa is used for human consumption referring to its importance as a staple and sustainable crop in that part of the world (CIP, 2008). This highly nutritious crop gives better and faster production under diverse agro-ecological conditions with less input (Lim et al., 2007) and has immense potential to combat food shortage, malnutrition and poverty (CIP, 2008).

Despite the added advantages of production and nutrition of sweet potato, its production is affected by various biotic and abiotic stress factors (Guo et al., 2006). Soil salinity is one of those factors that limit sweet potato productivity and expansion of cultivation in many parts of world including Africa where it is a staple food (Dasgupta et al., 2008). Sweet potato varietal improvement against salinity stress is necessary to improve its potential as a food security crop.

Genetic variation is the source for plant breeding and biotechnology provides an easy access to innovative genetic variation to support breeding. Tissue culture is the most potent part of biotechnology and is mainly employed in sweet potato germplasm maintenance, besides the production of somaclonal variants and the development of transgenic plants (Prakash, 1994; Sihachakr and Ducreux, 1987; Dodds et al., 1991). However, tissue culture is associated with somaclonal variation, that is, genetic variation induced during *in vitro* culture (Larkin and Scowcroft, 1981; Karp, 1995). Somaclonal variation can be assessed by the analysis of phenotype, chromosome number and structure, proteins, or direct DNA evaluation of plants (De Klerk, 1990; Jain, 2001; Smy´kal et al., 2007).

*Corresponding author. E-mail: nadiaanwerpk@yahoo.com.

Nevertheless, somaclonal variation is undesirable in some of the tissue culture based techniques such as *in vitro* propagation and genetic transformation where genetic stability of regenerable culture is required (Jain et al., 1998). However, somaclonal variation also could provide a natural source of variability and have been used for successful selection of novel cultivars in many plant species, particularly in vegetatively propagated crops (Heinz and Mee, 1971; Cote et al., 1993; Hedi and Bridgen, 1996; Hammerschlag et al., 2006). Despite the limitations associated with the use of somaclonal variation for selection of desired traits, it could provide a cost effective and easily accessible strategy for sweet potato improvement in areas where transgenic technology has resource limitations. There is no much work done on the identification and production of salinity tolerant sweet potato plants (Ekanayake and Dodds, 1993; Mukherjee, 2001) and somaclonal variation could be used for the selection of salinity tolerant sweet potato plants.

Sweet potato exhibit high rate of somaclonal variation (Zhang et al., 1997) and many researchers reported the existence of tissue culture derived polymorphism in this crop (Villordon et al., 1996; Lo et al., 2004; Lin et al., 2009). However, despite the importance of tissue culture derived variation in sweet potato, reports on the application of somaclonal variation in sweet potato are limited. A sweet potato cultivar 'Scarlet' was selected from shoot-tip culture-derived clones that is comparable to the parent cultivar in yield and disease resistance, but shows darker and more stable skin colour, which is a desirable quality trait (Moyer and Collins, 1983).

Sweet potato is notoriously "plastic" for high somatic mutation rates (Hernandez et al., 1964; LaBonte et al., 2000) and *in vitro* culture may further accentuate somaclonal variation in this crop. The studies on understanding and application of somaclonal variations in sweet potato are important as they may help in shaping the breeding strategies for its improvement. In the present study, we tried to evaluate the degree of somaclonal variation in regeneration via somatic embryogenesis by the phenoltype under salinity stress condition and to assess the potential of somaclonal variation for development of salinity tolerant cultivar in sweet potato.

MATERIALS AND METHODS

Production and culture of regenerated plants

We used one of the most widely used sweet potato cultivar in Japan named "Shiroyutaka" in the present study. "Shiroyutaka" is famous for its high starch yield but is sensitive to salt stress. "Shiroyutaka" plants were cultured on growth regulator free LS (Linsmaier and Skoog, 1965) medium for three weeks and then the leaf, petiole and stem explants were used for regeneration through somatic embryogenesis. Embryogenic calli were induced on LS medium containing 1 mg/ml of 4FA, 3% w/v sucrose and 0.32% w/v gellan gum. After 60 days of culture, embryogenic calli were transferred to somatic embryo formation media (LS medium, 4 mg/l of ABA, 1 mg/l of GA3, 3% w/v sucrose and 0.32% w/v gellan gum) for about 3 weeks in the dark. The generated somatic embryos were transferred to the plant formation medium (LS medium, 0.2 mg/l of zeatin riboside, 0.05 mg/l of ABA, 1.5% sucrose and 0.25% gellan gum) and cultured thereby, until the regenerates were appeared in the light length of 16 h. The regenerated plantlets were maintained in culture medium at 26°C with a 16 h photoperiod.

The 14 lines of regenerated plants (#1, # 2, #4, #5, #6, #7, #8, #9, #10, #12, #14, #17, #18 and #19) were maintained *in vitro* for subsequent evaluation of salinity stress tolerance. The non-regenerated "Shiroyutaka" original donor plants were used as control in this study. The vine cuttings of pot grown regenerated and control plants were collected and the explants were then subcultured before subjecting to phenotypic evaluation test through shoot apex culture.

Phenotypic evaluation by *in vitro* salinity screen system

To evaluate salt tolerance of sweet potato, an *in vitro* salinity screen system was established by a preliminary experiment using control plants. In the process of calibration, eight salt concentrations (0, 25, 50, 75, 100, 125, 150 and 200 mM) were applied to media and five plants per treatment were used in three repeated tests. The data was recorded at 3 - 4 days interval over a period of 27 days after inoculation (DAI) on growth parameters that were number on roots per plant, total root length, leaf and root condition by visual scoring. Total root length was sum of all roots that estimated through the culture tube. After 16 DAI, it was difficult to estimate root length. In visual scoring for leaf condition, plants were rated on a scale from 1 - 5 (Figure 1a), where 5 = green and healthy, 4 = pale, 3 = yellow or pale and injured, 2 = yellow and injured, 1 = died. In visual scoring for root condition, 1 - 5 scale was used (Figure 1b) where 5 = thick roots with lateral roots, 4 = thin and without lateral roots, 3 = stale but long, 4 = stale and very short, 5 = died or no roots.

Statistical analysis

All data were subjected to statistical analysis using factorial analysis of variance (ANOVA) based on randomized complete block design (RCBD) and the mean values obtained from the treatments were compared using Duncan's multiple range test (Duncan, 1955) at the 5% least squares regression (LSR) level using MSTAT-C statistical software.

RESULTS AND DISCUSSION

Establishment of an *in vitro* salinity screen system for sweet potato

Phenotypic evaluation based on morphological and physiological traits is one of the most popular methods for evaluation of somaclonal variations. We evaluated the phenotype of sweet potato regenerants obtained through somatic embryogenesis, under salinity stress conditions to screen not only for occurrence of somaclonal variation but also for identifying the potential variants with high salinity tolerance. To assess somaclonal variation by salinity tolerance screen, we developed a successful and reliable *in vitro* salinity screen system for sweet potato. *In vitro* system is considered as an ideal system for selection of salt tolerant plants, as it can be carried out under controlled conditions with limited space and time

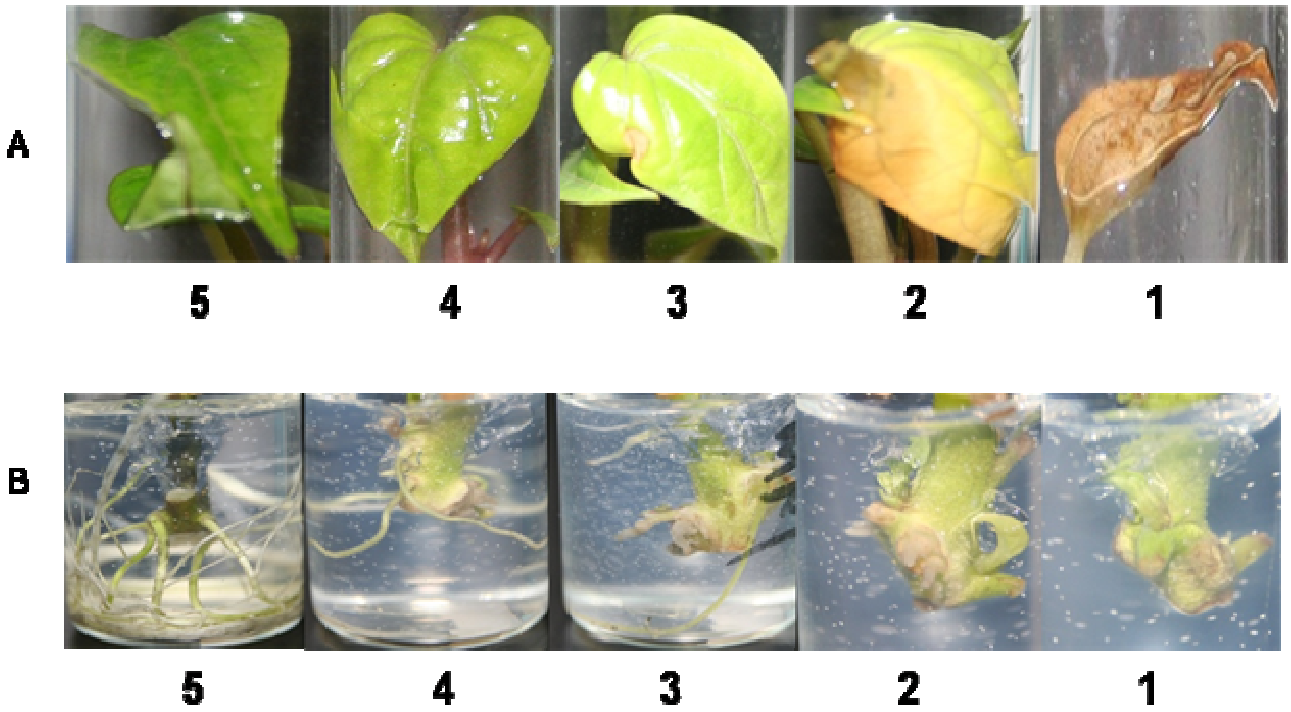

Figure 1. Representative example of visual scoring scale for leaf and root condition under *in vitro* salinity conditions. (A) Visual scoring scale for leaf condition, where 5 = green and healthy, 4 = pale, 3 = yellow or pale and injured, 2 = yellow and injured, 1= died. (B) Visual scoring scale for root condition where 5 = thick roots with lateral roots, 4 = thin and without lateral roots, 3 = stale but long, 4 = stale and very short, 5 = died or no roots.

(Gosal and Bajaj, 1984). The shoot apex culture has been used effectively for salinity tolerance assessment of various plant species including sweet potato (Chandler et al., 1988; Martinez et al., 1996; Cano et al., 1998; Dasgupta et al., 2008). We made three repeated tests using control plants as explained in material and methods section to develop such system for sweet potato.

The data was recorded at 3 - 4 days interval over a period of 27 DAI on growth parameters that were number on roots per plant, total root length, leaf and root condition by visual scoring under various NaCl concentrations (data not shown). The score of leaf and root condition began to decrease over 13 DAI. The tremendous increase in plant root length under lower NaCl conditions made it difficult to record data after 16 DAI. The duration of salt treatment was determined to be 16 DAI for convenient and efficient screening. Figure 2 summarizes all the four parameters at 16 DAI. As the plants response to salinity range below 100 mM was not very different from the non-saline condition (0 mM), 75 mM was selected to show that plant response to lower salinity conditions. On the other hand, a gradual negative effect was observed on all parameters after 75 mM and among others, 150 mM was shown to have a more distinct and stable pattern that can easily distinguish the salt tolerant plant than any other higher NaCl concentration. Therefore, 150 mM was chosen to estimate the plant salinity tolerance. Among all NaCl concentrations applied, 200 mM was found to be capable of effective assessment of plant survivability under high salinity, as shown by plants response in all the studied parameters.

Phenotypic evaluation by *in vitro* salinity screen system

We used 14 regenerated lines obtained through somatic embryogenesis and their performance was compared with control plant under *in vitro* salinity conditions. Sweet potato is considered recalcitrant in terms of regeneration (Sihachakr et al., 1997) which may contribute to the use of relatively small number of regenerated lines in the present study. The analysis of variance for growth parameters of *in vitro* (Table 1) indicated that, there were significant effects of treatments for all the studied parameters. Figure 3 shows the overall trend in the data obtained in the experiment on the performance of the regenerated and control plants.

A wide variation in the studied parameters was observed among the plants tested with respect to different levels of salinity (Figure 3). The results revealed that the studied parameters decreased significantly with increase in salinity stress. Plantlet growth was reduced remarkably in all the lines under 150 and 200 mM NaCl stress, compared with their performance on media supplemented with 0 and 75 mM salt concentrations. Our results are in

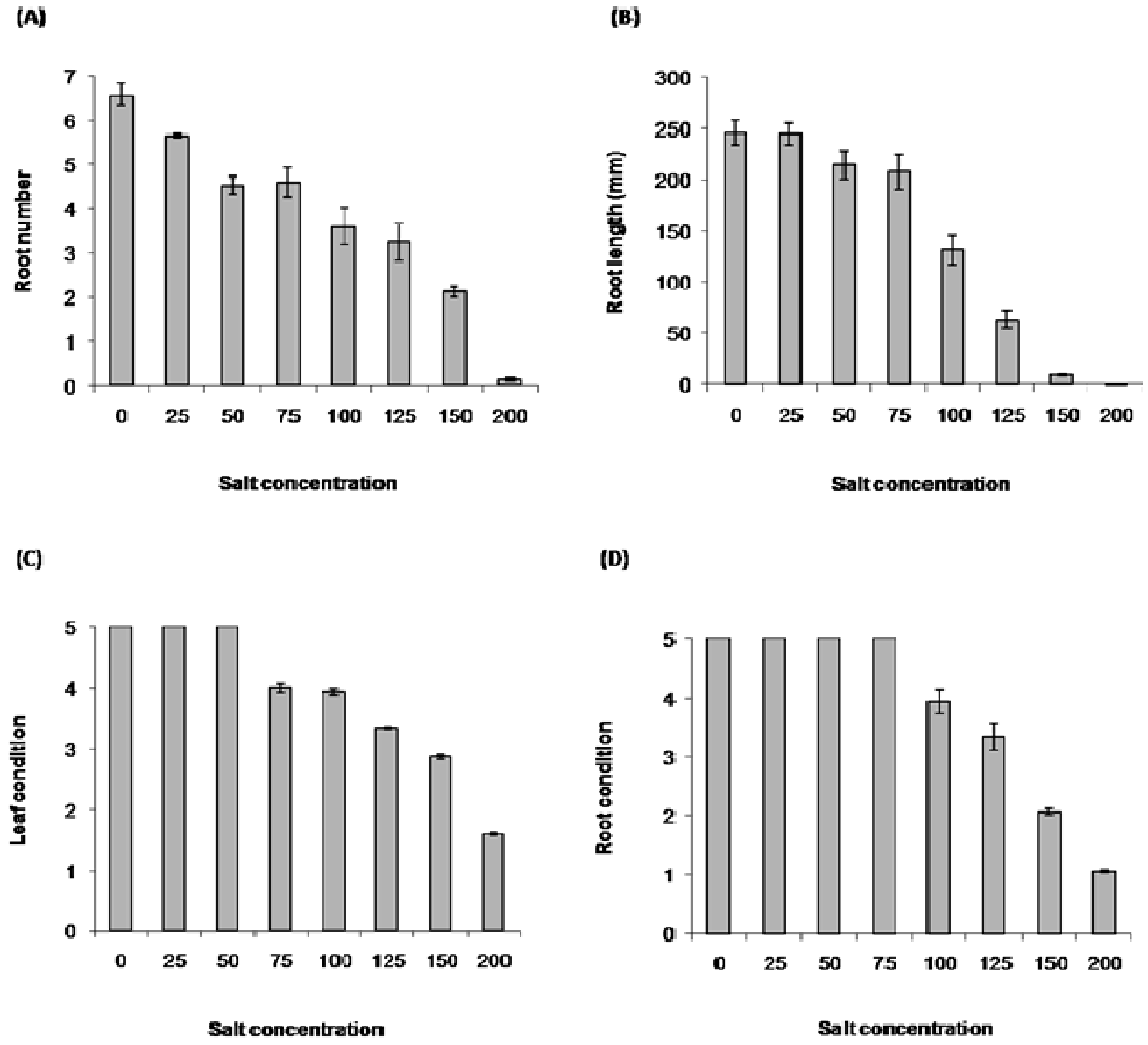

Figure 2. Optimization of conditions for *in vitro* salinity screen system using non-regenerated control plants. The abscissa refers to different NaCl concentrations supplemented in media (0-200 mM). The ordinate shows the average root number (A), total root length (B), average score for leaf condition (C) and average score for root condition (D). The data was recorded in three repeat tests at 16 DAI. The lines at the top of each bar indicate the respective standard error.

Table 1. ANOVA (mean sum of squares) for different parameters *in vitro* from factorial experiment in randomized complete block design.

Source	df	Parameters			
		Root number	Root length	Leaf condition	Root condition
Replication	2	12.387**	37689.581**	0.009	0.163**
Generation (lines)	14	5.248**	5869.463**	0.048**	0.244**
Salinity	3	319.380**	1073593.526**	9.324**	176.951**
Genotype x salinity	42	2.230**	2691.824**	0.047**	0.181**
Error	118	0.752	1801.305	0.018	0.025

** Significant at $p< 0.01$.

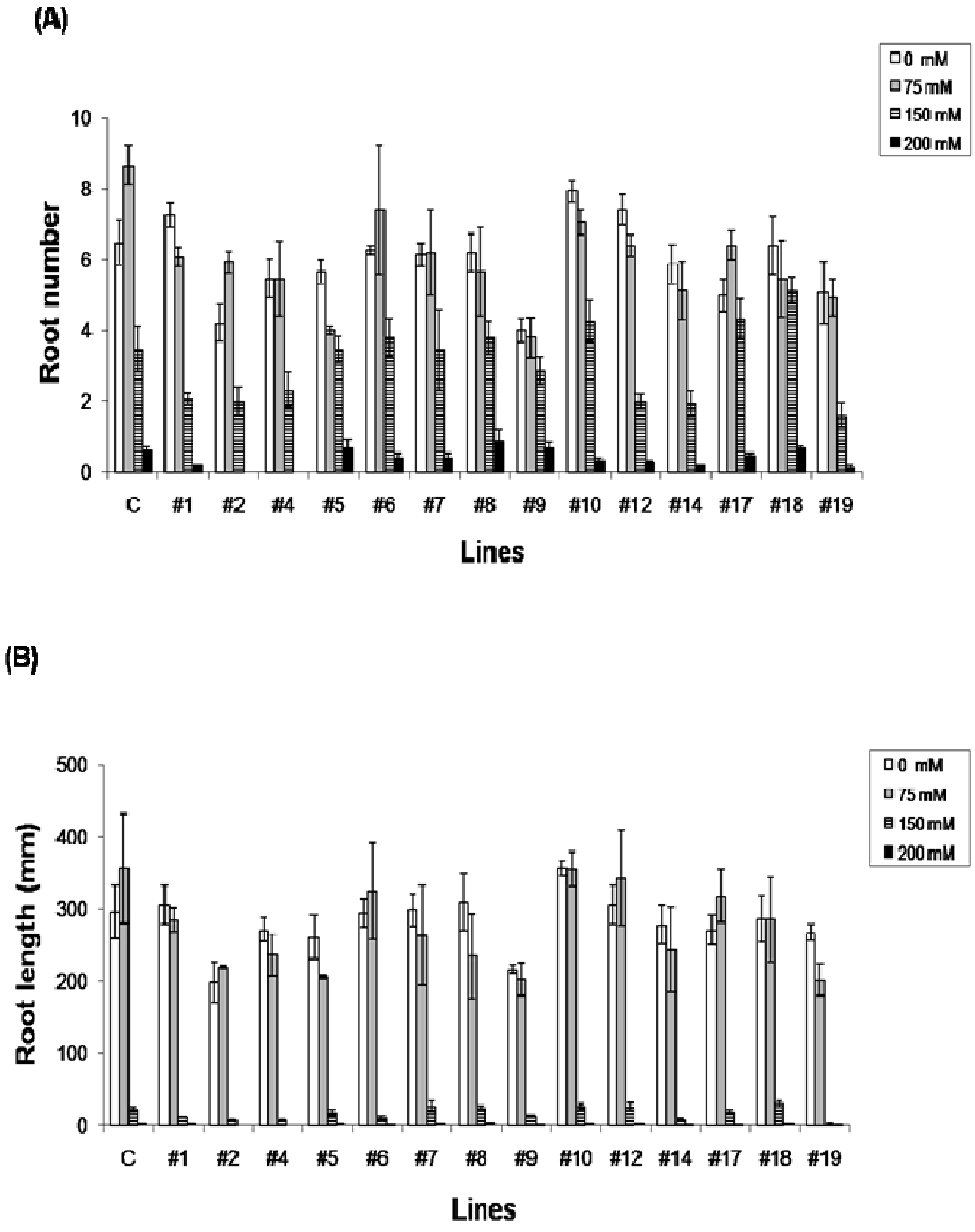

Figure 3. Plants response to different salinity levels under *in vitro* conditions. The abscissa refers to different regenerated lines (#1-19), with non-regenerated cv. Shiroyutaka as a control 'C'. The ordinate shows the average root number (A), total root length (B), average score for leaf condition (C) and average score for root condition (D). The lines at the top of each bar indicate the respective standard error.

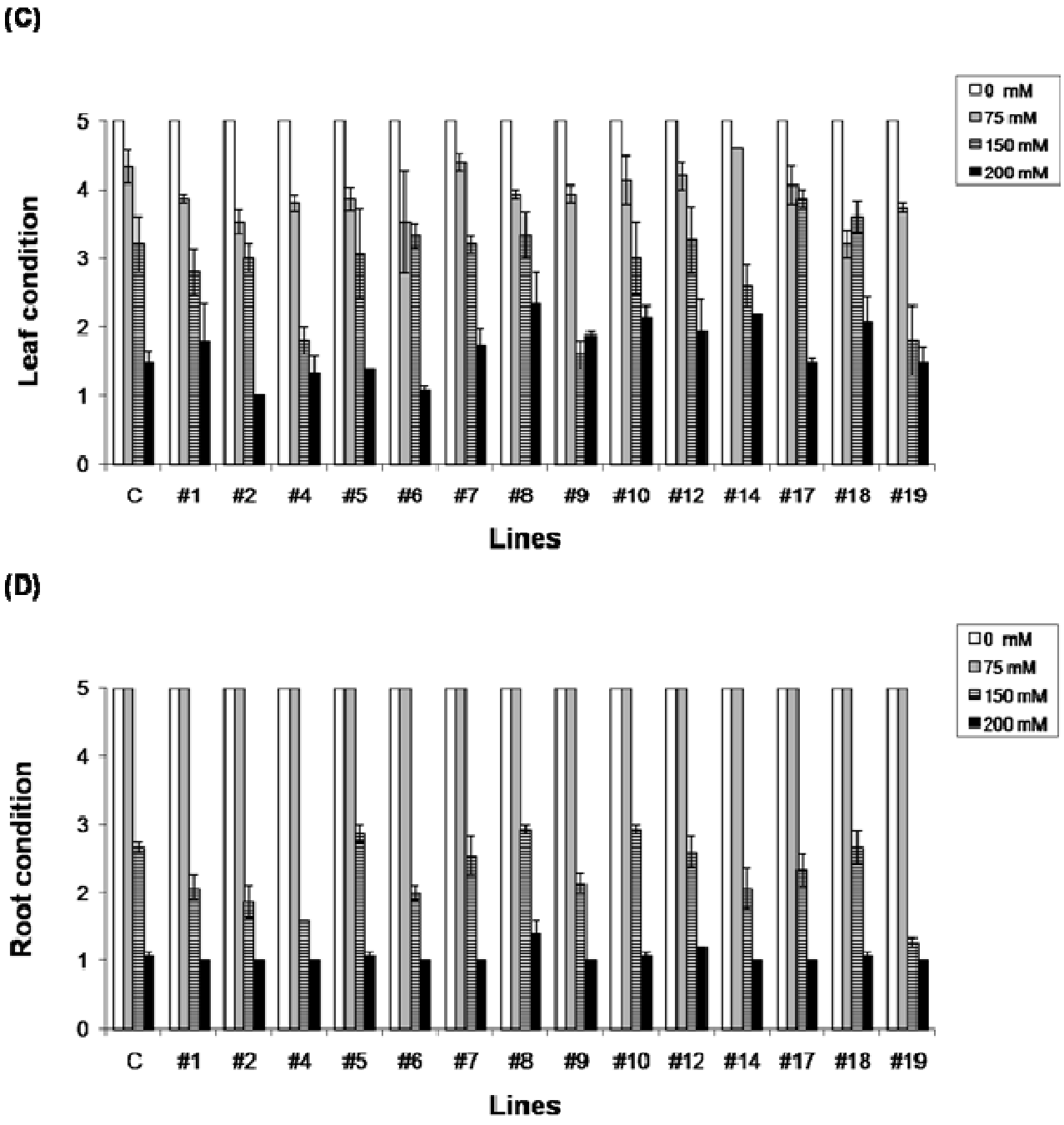

Figure 3. Continued.

accordance with other reports on sweet potato where drastic decrease in growth parameters was observed on increasing NaCl concentration from 0.5 - 1% under *in vitro* conditions (Mukherjee, 2001; Dasgupta et al., 2008). Rooting parameters were affected more prominently than leaves, which could be attributed to the over-sensitivity and direct contact of roots to salinity conditions (Martinez et al., 1996; Cano et al., 1998; Mercado et al., 2000; Vijayan et al., 2003; Dasgupta et al., 2008). As a whole, survivability of the explants was reduced largely on 200 mM NaCl that may be considered for stringent selection in stress tolerance studies in sweet potato (Luan et al., 2007).

Comparison of salinity tolerance by Duncan test

As ANOVA for phenotypic evaluation of salinity stress

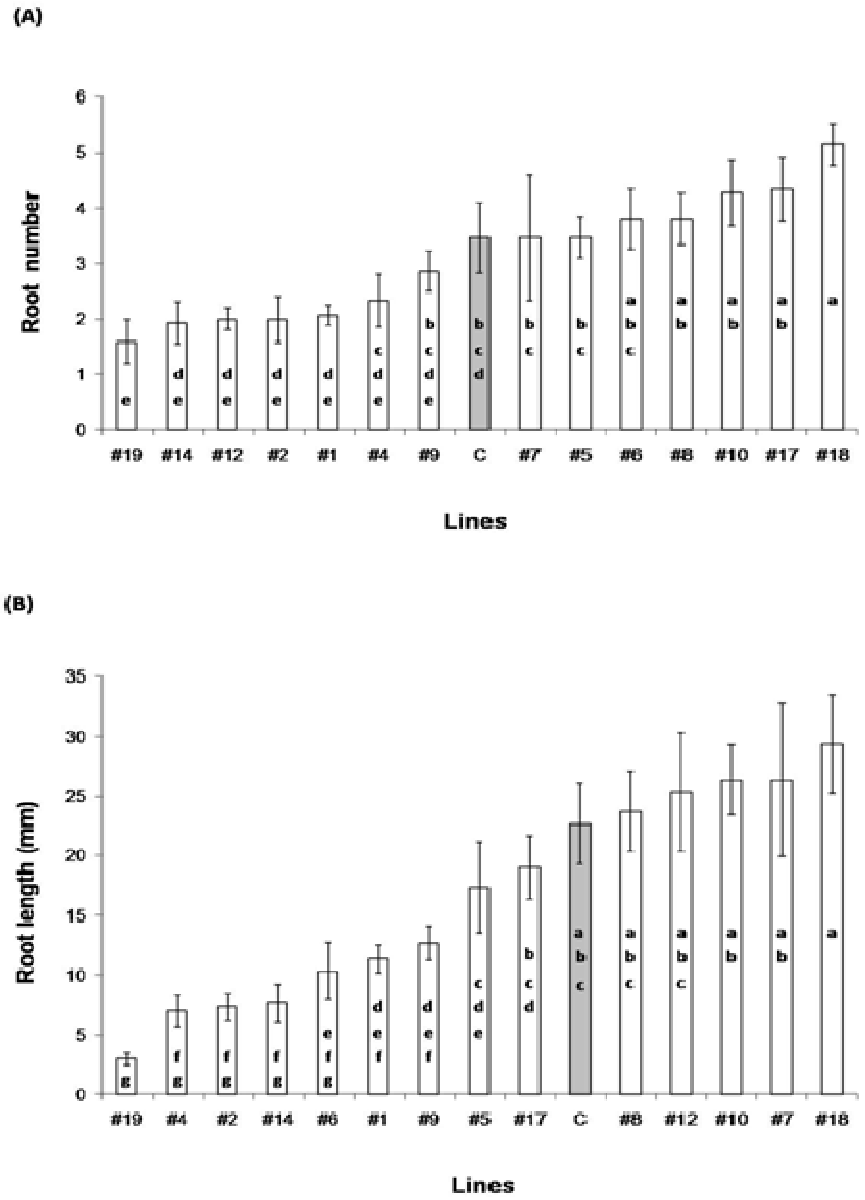

Figure 4. Comparison of performance of regenerated and control lines for different parameters under *in vitro* salinity stress using Duncan test at LSR level of 5%. Assessment of salinity tolerance in regenerated and control lines, with mean values of five cuttings from three repetition of salinity test. The abscissa refers to different regenerated lines (#1-19), with non-regenerated cv. Shiroyutaka as a control 'C'. The ordinate shows the average root number (A), total root length (B), average score for leaf condition (C) and average score for root condition (D) under 150 mM salinity condition. The bar for each line shows the mean value for the corresponding parameter. The lines at the top of each bar indicate the respective standard error. The letters 'a' to 'g' are used to divide the plants into recognizable groups.

tolerance showed significant generation effect (Table 1) therefore, to evaluate the extent of variation in terms of salt tolerance among the regenerated and control lines, Duncan's multiple range tests was used. We used 150 mM salinity condition as the effective concentration for salinity tolerance evaluation to compare the performance of the regenerated and control lines. The regenerated and control lines were grouped based on Duncan's test under 150 mM conditions and the related means are shown as a bar graph (Figure 4). In Duncan's test classification, a typical grouping would be between 'a' to 'g' where 'a' is the group of most tolerant to salinity stress

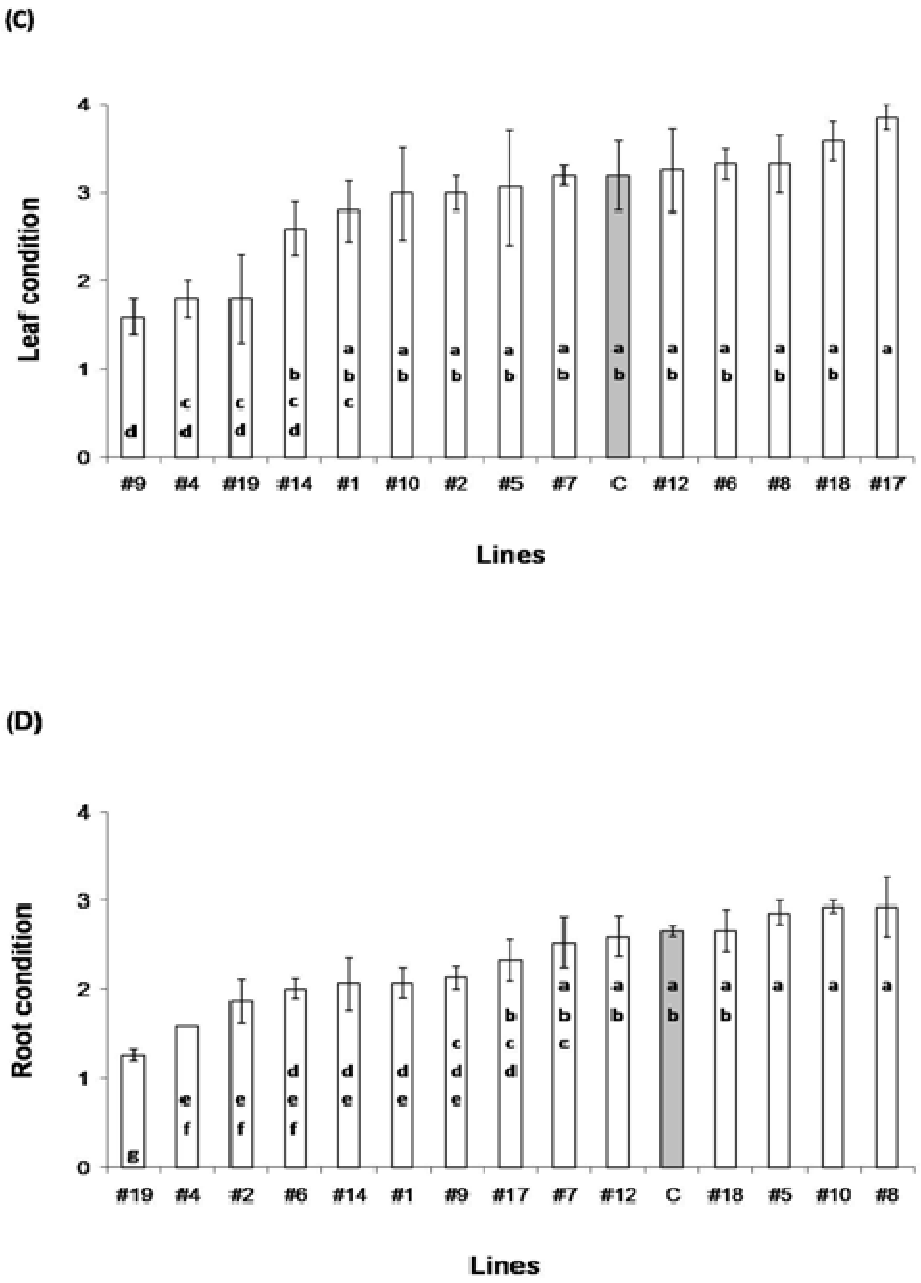

Figure 4. Continued.

and ‘g’ is the most sensitive.

Among the regenerated lines, a wide range of significant variation was observed for all the parameters in regenerated plants compared to control plants (Figure 4). For root number, control line categorized in group ‘bcd’. About 86% of the regenerated lines (group ‘ab’ to ‘de’) fell within the same salinity tolerance range as of control plants while the rest of two lines were found to be significantly different from the control lines (group ‘a’ and ‘e’) (Figure 4a). The mean value of root number for regenerated line #18 (group ‘a’) was significantly higher than the control and most of the regenerated lines. The regenerated line #19 in group ‘e’ showed significantly low mean value of root number than the control and most of the regenerated lines. In case of comparison among regenerated and control lines for root length, the half of regenerated lines (group ‘a’ to ‘cde’) fell within the same salinity tolerance range as control plants while the rest seven regenerants were found to be significantly different from the control lines (group ‘def’ to ‘f’) (Figure 4b). All the significantly different variants in regenerated lines were found to be low in tolerance to salinity stress than control

and other regenerated lines. Similarly, by comparing leaf and root condition scores between regenerated and control plants, 21% (group 'cd' to 'd') (Figure 4c) and 50% (group 'cde' to 'g') (Figure 4d) of regenerants were found to be significantly different from the control lines, respectively. All the significantly different variants in regenerated lines were found to be low in tolerance to salinity stress than control and other regenerated lines.

As a whole, it was shown that significant line differences exist between regenerated and control plants in root number, root length, leaf and root condition. The observed variation in performance of regenerated and control plants under salinity stress condition showed the presence of somaclonal variation among the callus derived plants. The substantial existence of phenotypic variation in regenerated plants might have been caused by prolonged physiological disturbances occurred during tissue culture.

The successful application of somaclonal variation to improve plant species for desired traits depends on the rate and type of somaclonal variation obtained. In general, most of the somaclonal variations are with negative effects (Morishita and Yamada, 1981; Morishita, 1991). In the present study, the decreased mean performance of some of the variants for studied traits showed the negative effects of tissue culture derived variation in sweet potato. However, the identification of a variant with average increase in performance showed the positive effects.

Plant genotype and regeneration method are considered as the most influential factors affecting rate of somaclonal variation (Ezura et al., 1995). Different types of cultured tissues are ranked in order of low to high genetic instability as follows: micropropagation from isolated shoot tips and meristem, adventitious shoot formation, somatic embryogenesis, and organogenesis from callus, cells, and protoplasts (Damasco et al., 1996). The phenotypic assessment of somaclonal variations in regenerants obtained from somatic embryogenesis of sweet potato explants also gives an estimate of fidelity of regeneration method for application where high genetic stability is concerned.

In the present study, despite the fact that none of regenerated plants showed improved salt tolerance, the variability among the *in vitro* regenerated plants shows the potential of callus culture for creating genotypic variability and subsequent selection for desirable traits in sweet potato. Moreover, the assessment of somaclonal variation from source culture point of view gives an estimate of feasibility of somatic embryogenesis for use in plant transformation and other applications.

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